

Sex But Not Altitude, Modulates Phenotypic Covariations Between Growth and Physiological Traits in Adult Asiatic Toads

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Abstract The pace-of-life syndrome (POLS) hypothesis predicts that most variation in life history, physiology, and behavior among individuals, populations, and species falls along a continuum from slow to fast pace of life. While there is evidence for climatic gradient-mediated POLS patterns among species, this approach has rarely been explicitly used to study POLS patterns among- and within- populations. In addition, the roles of sex in POLS evolution among- or within- populations are largely unknown. In this study, we investigated the effects of altitudinal gradient and sex on the covariations between growth rate and several physiological traits closely associated with POLS (blood glucose, baseline- and stress-induced glucocorticoids (GCs), hemolysis and hemagglutination) in the Asiatic toad *Bufo gargarizans*. Contrary to our expectation, altitudinal gradient had no influence on the covariations between growth rate and physiological traits, neither at the among- nor within- population level, indicating that these trait integrations have similar fitness payoffs across hierarchical levels. In contrast, we found evidence for sex-specific POLS composition: there was a negative covariance structure between growth rate and baseline GCs- but only in females, and a positive covariance structure between

growth rate and baseline GCs- but only in females, and a positive covariance structure between growth rate and hemagglutination-but only in males. This observation indicates that these trait associations differ dramatically in advancing fitness for each sex, and supports the idea that sex-specific POLS composition could evolve in species in which the reproductive roles largely differ between the sexes.

Keywords *Bufo gargarizans*, constraint, glucocorticoids, immunity, metabolism, phenotypic integration, physiological pace-of-life syndrome

1. Introduction

Life history theory posits that under limiting resources, the trade-off between allocation to current versus future reproduction or survival can generate a correlative pattern between life history traits, resulting in a slow-fast life history continuum (Stearns, 1989). Recently, researchers further proposed that life-history characteristics and suites of physiological (metabolic, immunological and hormonal) and behavioral traits have coevolved in response to environmental conditions forming a pace-of-life syndrome (POLS) (Dammhahn *et al.*, 2018; Montiglio *et al.*, 2018; Careau *et al.*, 2008; West-Eberhard, 2003; Klingenberg, 2014; Ricklefs and Wikelski, 2002; Speakman, 2005; Tomasek *et al.*, 2019). This functional integration among multiple phenotypic traits is

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primarily caused by correlational selection, and such processes have important consequences for the evolutionary and ecological study of populations (Tieleman *et al.*, 2005; Réale *et al.*, 2010).

Due to global climate change, there is increasing interest in the intraspecific local adaptation of populations along geographical gradients (Tieleman *et al.*, 2006; Pettersen, 2020). Populations generally show different life-history and physiological traits across latitude/altitude ranges that result from distinct selective regimes (Conover *et al.*, 2009). Therefore, consistent differences in population trait means are anticipated, forming in a POLS across latitude/altitudinal gradients. Nevertheless, while there is evidence for latitude/altitude-mediated POLS patterns among species (Tieleman *et al.*, 2006; Wiersma *et al.*, 2007; Londono *et al.*, 2015), this approach has rarely been explicitly used to study POLS patterns among- or within- populations of the same species. The few studies looking at differences in within-population syndrome structure across different environmental gradients revealed large similarities of covariation patterns, however these studies were limited to behaviors (Segev *et al.*, 2017; Alcalay *et al.*, 2015; Bengston and Dornhaus, 2015; Pruitt *et al.*, 2008; Debecker and Stoks, 2019; Brans *et al.*, 2018). Considering the distinct roles of physiology and behavior in life history evolution (Polverino *et al.*, 2018), whether these covariation patterns across geographic gradients also hold for physiology remains a significant gap in our knowledge.

Sex-specific optima for reproductive investment and life history scheduling could result in sex differences in mean trait expression (Wedell *et al.*, 2006). Similarly, as a result of their different reproductive roles and the environment, each sex may come to differ in the strength of correlation among traits, or different traits may covary in males and females. According to the source of sex-specific selection on pace of life and trait covariances, four conceptual frameworks have been proposed: uniform POLS structure, sex-specific POLS with different strength trait correlation, distinct POLS structure in each sex, no POLS (Hamalainen *et al.*, 2018). For instance, uniform POLS would be expected when sexual conflict is either completely unresolved- such that high genetic correlations between the sexes prevents the evolution of dimorphism, or when sexual conflict is absent and therefore the life history strategies of the sexes converge. However, classic evidence for these outcomes is presently scarce.

In this study, we examined how metabolic, immunological, or hormonal traits covary with one life history trait – growth – as well as the role of altitudes and sexes in the among- and within populational covariations in the Asiatic toad (*Bufo gargarizans*) (Ricklefs and Wikelski, 2002). The distribution range of this species spans an extremely large altitudinal

gradient from zero to 4300 m above sea level (Fei, 2009). Their life history traits also display significant altitudinal differences (Liao and Lu, 2012; Liao *et al.*, 2014; Guo *et al.*, 2016), making this species suitable for studies related to climatic gradient-mediated or sex-specific POLS. We measured rate of growth, baseline blood glucose concentrations (G0), two immunological indices (hemagglutination, hemolysis), and glucocorticoids (GCs, baseline/stress-induced corticosterone) in a common garden setting. As a major blood-borne source of metabolizable energy circulating in animal blood, G0 may correlate with energy-demanding processes such as growth and reproduction (Fournier *et al.*, 1992; Tomasek *et al.*, 2019). For innate immunity characteristics, we measured hemolysis and agglutination since they are more dependent on genetic background than adaptive immunity (Tieleman *et al.*, 2005). We measured GC concentration because of its functional relationship with immunity and energy mobilization (Landys *et al.*, 2006). Many studies measuring GC levels have found variable associations with reproductive traits in fish, amphibians and reptiles (Crespi *et al.*, 2013).

In accordance with the POLS hypothesis and the recent POLS-related theoretical framework, we expected a faster physiological pace-of-life in *B. gargarizans* that show higher G0 and lower GCs and innate immunological indices, and are more likely to persist at lower altitudes or in males (Réale *et al.*, 2010; Hamalainen *et al.*, 2018). We postulated that covariations should be formed between growth and physiological traits, which are expected to be more pronounced at lower altitudes because warmer and more productive environments can bring up these associations (Segev *et al.*, 2017; van Noordwijk and de Jong, 1986). We also hypothesized that *B. gargarizans* demonstrates sex-specific POLS. However, we were not able to predict which sex would display the stronger covariance structures due to lack of a *priori* knowledge on how these physiological traits confer to breeding an advantage in this species.

2. Materials and methods

2.1. Sampling and acclimation procedure A total of 123 adult males were collected from seven sites in western China in summer 2018 and spring 2019. These sites were divided into two gradient groups according to their relative altitude (group L<~1000m, group H>1000m, Figure S1 and Table 1). Sites 1–4 form the first transect of the Min River drainage, and sites 5–7 form the second transect along the Dadu River drainage. After sampling, all individuals were transferred to the animal room in Chengdu Institute of Biology, Chinese Academy of Sciences (CIBCAS). A constant temperature (20±1°C and light/dark cycle (12h:12h) was maintained in the

animal room. All toads were housed individually in a plastic container (35.5cm×25cm×15cm, L×W×H) that included a piece of wet sponge (5cm×7cm) to preserve humidity and a U shape tile (15cm×14cm×7cm) for shelter. The toads were mainly fed mealworms (*Tenebrio molitor*) dusted with calcium powder (Exo Terra). To ensure the toads could intake fresh mealworms *ad libitum*, the food plate was cleared and replenished every other day. Live crickets (*Gryllus rubens*) were also supplied once a week. We measured the body mass and snout-vent length (SVL) once every month.

After captive acclimation of more than 4 months, each toad received intraperitoneal injections of a 50 μ L mixture of the keyhole limpet hemocyanin (KLH, 2 mg/25 mL, Enzo#ALX-202-064-M025) and immunological adjuvants (Sigma#F5881) (day 0). Two weeks later, all individuals were injected with 25 μ L of a KLH solution again to further boost the immune system (we were not able to acquire those data in the Asiatic toads due to the low specification of the designed antibody, anti-toad IgY Ab). At day 56, we collected saliva samples for a corticosterone assay. At day 63, we sacrificed all the toads with 0.2% ms-222 solution and collected plasma by opening the abdominal cavity and drawing blood from the opening of the right ventricular cavity with a heparin-rinsed syringe. The blood sample was centrifuged at 3000 rpm at 4°C for 5 min after chilling on ice for <1 h. The upper plasma was carefully separated and frozen for further analyses. All experimental procedures followed the guidelines of the Animal Care and Use Committee of CIBCAS.

2.2. Baseline and stress-induced corticosterone levels To determine the baseline corticosterone content, we collected saliva samples. A small, dry cotton ball of known weight was inserted in the toad's mouth for 20 s; all samples were collected within 3 minutes in order to minimize the increase in corticosterone due to treatment stress (Davis and Maney, 2018). To determine stress-induced corticosterone concentrations, target toads were placed in cloth bags for 60 min, and the

second saliva sample was taken thereafter. We examined corticosterone content in saliva followed the method proposed by (Janin *et al.*, 2012). We weighed the saliva-soaked cotton balls and stored them individually at -80°C. To exact corticosterone from the saliva samples, we added 1 mL methanol to each sample tube for 5 h, transferred the cotton ball and methanol to a centrifuge tube with a filter membrane and centrifuged at 8000 rpm at 4°C for 5 min. The collected solutions were concentrated with a vacuum centrifuge for 4 h at room temperature. The dry pellets were dissolved in 60 μ L pure water for radioimmunoassay. The extracted corticosterone samples were measured with Iodine [125I]- Rabbit- Cortisol Radioimmunoassay Kit (Beijing North Institute of Biological Technology, China). Fetal bovine serum was used as the intraplate control. The intra-assay coefficient of variation (CV) was <10% and inter-assay CV was <15%. The lower and upper effective limits of the corticosterone assay kit were between 10 ng/mL and 500 ng/mL, respectively.

2.3. Baseline blood glucose content The glucose concentration in plasma was determined by a glucose oxidase assay kit (Applygen, E1010). Firstly, we diluted the plasma samples three folds with PBS buffer, then added 10 μ L of the standard glucose solution (2000, 1000, 500, 250, 125, 62.5, 31.25, 15.625 μ mol/L) or plasma sample, and 190 μ L of the working solution to the 96-well plate. After incubation at 37°C for 20 min, we measured the absorbance value at 550 nm with an Enzyme standard instrument (Thermo Scientific Varioskan Flash) and calculated the glucose content based on the standard curve.

2.4. Indices of constitutive immunity Complement and natural antibody levels were measured using an erythrocyte hemolysis- hemagglutination assay (Matson *et al.*, 2005; Sparkman and Palacios, 2009). Serial two-fold dilutions of 50 μ L plasma were made with phosphate-buffered saline (PBS) in 96-well (8 rows×12 columns) round (U) bottom assay plates. Each well received 50 μ L of a rabbit red blood (1%) cell

Table 1 Sample site and sample size information.

Site	Gradient	Altitude (m)	Longitude (°)	Latitude (°)	Sample size
Min River drainage					
1. Yingxiu	L	828	103.4532	31.0348	12♂9♀
2. Gengda	H	1629	103.3241	31.1058	14♂13♀
3. Wolong	H	2053	102.9785	30.863	16♂14♀
4. Dengsheng	H	2689	102.3777	30.863	4♂4♀
Dadu River drainage					
5. Shimian	L	926	102.3777	29.2567	19♂
6. Hailuogou	H	1689	102.1129	29.6119	13♂
7. Kangding	H	3239	102.0426	29.8382	5♂

suspension. Plates were incubated for 60 min at 20°C and then scored. Titers were estimated as the negative log₂ of the highest dilution factor of plasma that showed hemagglutination or lysis. Half scores were given for titers that appeared intermediate. All the samples were assayed in duplicate with positive and negative controls in each plated.

2.5. Statistical analyses All statistical analyses were performed with IBM SPSS Statistics 21.0 software (International Business Machines Corporation) and R (Version 4.0.3). We calculated the growth rate as the body mass gain per day (g/d) during the experiment period. Four subjects died during the experiments and were hence excluded from the analysis. To achieve normality, blood glucose and corticosterone were transformed with Jonson transformation. Erythrocyte hemolysis and erythrocyte agglutination were computed with Blom rank-based normalization.

Since our experimental design was not balanced, we separated the data into two datasets and analyzed them with general linear models. Dataset I included all data on males (data from males in transect I and II), which was used to examine the effects of transect and altitude on growth, physiological variables and their covariations. Dataset II included the data of both sexes from transect I, and was used to examine the roles of sex and altitude in those phenotypic traits.

We used a univariate mixed model (UMM) to analyze the effects of altitude/transect or altitude/sex on growth and physiological variables using the “MCMCglmm” R package (Hadfield, 2010). For each UMM, altitude, transect (or sex), transect (or sex) and their two-way interaction (altitude×transect or altitude×sex) were included as fixed effects, SVL or body mass as covariates to control for the effects of age (initial body mass but SVL was used for growth rate, since SVL and initial mass were correlated and only initial body mass was covaried with growth rate). Sampling site was used as a random effect.

To investigate the covariation between growth rate and physiological variables at the among- and within-population level, we used a bivariate mixed model using the “MCMCglmm” R package (Hadfield, 2010). We first examined the variance and covariance matrix with covariates in the models. We then examined the changes in the variance and covariance matrix caused by the inclusion or exclusion of fixed factors (altitude, transect/sex) in the bivariate model. The response and explanatory variables were scaled before analysis (mean centered on 0 and SD reduced to 1) to facilitate the interpretation of the results. Sampling site was included as a random effect on all traits. We used the following priors for the residual ($V = \text{diag}(2)$, $\nu = 0.002$) and random effect matrices ($V = \text{diag}(2) \times 0.002$, $\nu = 1.002$, $\alpha.\mu = \text{rep}(0.2)$, $\alpha.V = \text{diag}(25^2, 2, 2)$). To compute the posterior

distribution, the model was run over 420 000 iterations, with a burn-in of 20 000 and a thinning interval of 100, to obtain an effective sample size between 20 001 and 419 901, with an autocorrelation level between retained iterations lower than 0.05.

3. Results

Initial body mass was positively associated with growth rate, and SVL was negatively associated with hemagglutination titer and stress-induced GCs levels ($P < 0.05$, Table S1–S2). Moreover, there were no significant differences among altitudinal groups in growth rate and physiological variables in either dataset ($P > 0.05$, Table S1–S2). Similarly, transect did not affect growth rate or physiological indices ($P > 0.05$, Table S1). However, growth rate and physiological variables (excluding stress-induced GCs) varied between the sexes, with females having higher growth rate, hemolysis and hemagglutination in females, and males having with higher baseline GCs, higher blood glucose content ($P < 0.05$, Figure 1, Table S2).

At the between-population, we did not find any significant covariance between growth rate and physiological variables (Table S3–S4). At the within-population level, growth rate significantly covaried with baseline GCs in both datasets (mean [95% credible interval]: $-0.334[-0.610, -0.100]$ for dataset I; $-0.568[-0.846, -0.323]$ for dataset II) (Table S3–S4). Moreover, growth rate significantly covaried with hemagglutination in both datasets ($0.256[0.038, 0.533]$ for dataset I; $0.472[0.230, 0.764]$ for dataset II) (Table S3–S4). These covariances were not modulated by transect or altitudinal group (Table S3–S4). Nevertheless, these covariances were significantly reduced when sex was included in the explained variables of MCMCglmm modeling in dataset II ($-0.247[-0.426, -0.081]$ for the combined growth rate and baseline GCs, $0.260[0.083, 0.473]$ for the combined growth rate and hemagglutination, Table 2). Further analyses showed that the covariance between growth rate and baseline GCs was significant in females (male: $-0.6[-1.562, 0.921]$, female: $-0.225[-0.477, -0.014]$, Figure 2), and the covariance between growth rate and hemagglutination was significant in males (male: $0.288[0.0629, 0.565]$, female: $0.149[-0.047, 0.390]$, Figure 2).

4. Discussion

4.1. Effects of altitude and sex on growth and physiological traits Our results demonstrate that the life history traits and physiological traits closely related with pace-of-life in *B. gargarizans* were similar along the altitudinal gradient. These similarities could be largely associated with the mixed effects

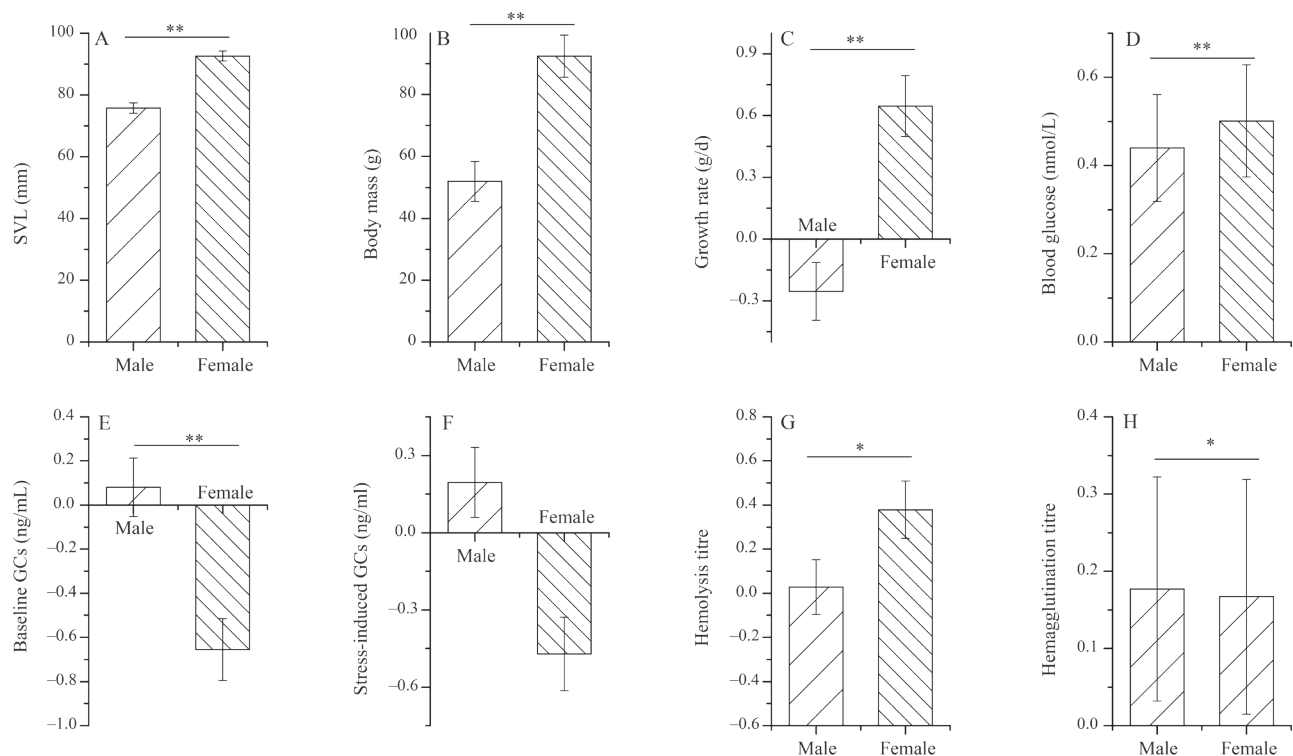


Figure 1 The effects of sex on snout-vent length (SVL) (A), body mass (B), growth rate (C), blood glucose (D), baseline corticosterone (E), stress-induced corticosterone (F), hemolysis (G), hemagglutination (H), of dataset II in *Bufo gargarizans*. The results are based on univariate mixed models (UMM) with altitude, sex, and their interaction as fixed effects, body mass (for growth rate) or SVL (for the other dependent variables) as covariates, and sampling site as a random effect. Data were presented as estimated mean values \pm standard error. Stars refer to significant differences between sexes (* $P < 0.05$, ** $P < 0.01$).

of genetic adaptation and phenotypic plasticity (Conover and Schultz, 1995). At high altitudes/latitudes, compensatory responses in life-history and physiological traits are expected to evolve, if the reductions in these phenotypic traits at low temperature involves a reduction in fitness, also known as counter-gradient variation (Conover and Schultz, 1995; Conover *et al.*, 2009). For instance, although tadpoles derived from cold environments tend to intrinsically grow faster than those derived from warm environments, tadpoles experiencing cold conditions at early-life stages grow slower than those experiencing warm conditions (Seebacher and Grigaltchik, 2014). Nevertheless, an earlier study revealed that the bufonids exhibit virtually no relation between size at metamorphosis and adult size (Werner, 1986). This decoupling suggests that maternal effects masking the genetic adaptation in the growth of *B. gargarizans* could emerge after metamorphosis.

It is noted that in addition to growth rate, the measured metabolic, immunological, hormonal indices of *B. gargarizans* differed significantly between the sexes, which could be associated with their sex-specific processes of ecology and evolution. The high rate of growth in females was traditionally

considered to be driven by sexual and fecundity selections, and substantially contributes to sexual size dimorphism (Chou *et al.*, 2016). The relatively low immunocompetence in males is likely to be associated with the immunosuppressive roles of testosterone content, which synchronously promote the development of sexual signals (Folstad and Karter, 1992). In addition, the relatively high baseline GCs and blood glucose content in males may indicate that the males' maintenance energy expenditure (e.g. respiration, immuno-competency, blood circulation, digestion) is higher than females' (Tomasek *et al.*, 2019).

4.2. Covariation in growth rate and physiological traits among and within populations

The results of our study found strong phenotypic correlations between growth rate, and baseline GCs and hemagglutination, supporting POLS at the within-population level. However, these correlations were not found at the among-population level, indicating that the coupling between life-history and physiological traits may vary across hierarchical levels (Réale *et al.*, 2010). Since high baseline GCs level are suggested to indicate poor health condition, the negative correlation between growth rate and

Table 2 Estimates of fixed effects and variance components for growth rate and physiological traits in of dataset II, obtained from bivariate mixed models. Panel A reports the estimates of fixed effects and variance components only with covariates included in the explanatory variables, and panel B reports the variance components after including altitude into the explanatory variables, and panel C reports the variance components after including transect into the explanatory variables. Variation in response and explanatory variables were scaled before analysis to facilitate the interpretation of the estimates. Given the structure of the data, the residual variance and covariance are interpreted as the phenotypic (co)variance within populations. The table gives the mean posterior distribution and its 95% credible interval (CI).

		Growth rate		Baseline GCs	
		Estimate	95% CI	Estimate	95% CI
Panel A	Var _{population}	0.962	0, 1.421	0.641	0, 1.542
	Var _{residual}	0.997	0.713, 1.313	1.043	0.716, 1.399
	Covar _{population}	-0.062	-0.545, 0.245		
	Covar _{residual}	-0.568	-0.846, -0.323		
Panel B	Var _{population}	21.316	0, 27.827	44.385	0, 48.555
	Var _{residual}	0.998	0.673, 1.339	1.047	0.700, 1.404
	Covar _{population}	-0.763	-4.55, 4.684		
	Covar _{residual}	-0.568	-0.870, -0.299		
Panel C	Var _{population}	1.167	0, 4.052	1.188	0, 4.654
	Var _{residual}	0.562	0.377, 0.752	0.813	0.577, 1.094
	Covar _{population}	-0.258	-1.447, 1.037		
	Covar _{residual}	-0.247	-0.426, -0.081		
		Growth rate		Hemagglutination	
		Estimate	95% CI	Estimate	95% CI
Panel A	Var _{population}	0.653	0, 2.354	1.475	0, 4.931
	Var _{residual}	0.996	0.697, 1.280	0.962	0.652, 1.303
	Covar _{population}	0.083	-0.586, 1.092		
	Covar _{residual}	0.472	0.230, 0.764		
Panel B	Var _{population}	10.061	0, 42.757	24.355	0, 61.043
	Var _{residual}	0.983	0.691, 1.300	0.93	0.639, 1.244
	Covar _{population}	0.529	-6.136, 8.267		
	Covar _{residual}	0.448	0.204, 0.727		
Panel C	Var _{population}	1.962	0, 6.574	2.597	0, 8.527
	Var _{residual}	0.554	0.363, 0.753	0.863	0.599, 1.160
	Covar _{population}	0.392	-1.352, 3.222		
	Covar _{residual}	0.26	0.083, 0.473		

baseline GCs could suggest an allocation trade-off between growth and survival in the Asiatic toad (Lankford *et al.*, 2001; Kim *et al.*, 2011). Moreover, the positive correlation between growth rate and hemagglutination level in the Asiatic toad is congruent with the prediction of the POLS hypothesis that innate immunity is favored by fast pace-of-life (Lochmiller and Deerenberg, 2000; Sparkman and Palacios, 2009).

Although growth rate was integrated with baseline GCs and hemagglutination, their covariances did not change among or within- altitudinal gradient. This result does not support the prediction of POLS that trait integration is more likely to occur in warm environments, and these trait combinations have similar fitness payoffs (Reznick *et al.*, 2000;

van Noordwijk and de Jong, 1986). This result is similar to that reported in a recent study of *Ischnura elegans* damselfly larvae, which showed that latitude did not alter the covariation between life-history and anti-oxidative physiological traits (Debecker and Stoks, 2019). On the other hand, a study on the water flea *Daphnia magan* found that the covariation structures between life history and stress physiological traits changed along an urbanization gradient (Brans *et al.*, 2018). Therefore, it seems that the impacts of anthropologic disturbance on trait integration already outweigh those imposed by climatic gradients. Nevertheless, more investigations in different species and manipulation approaches are needed to confirm this scenario.

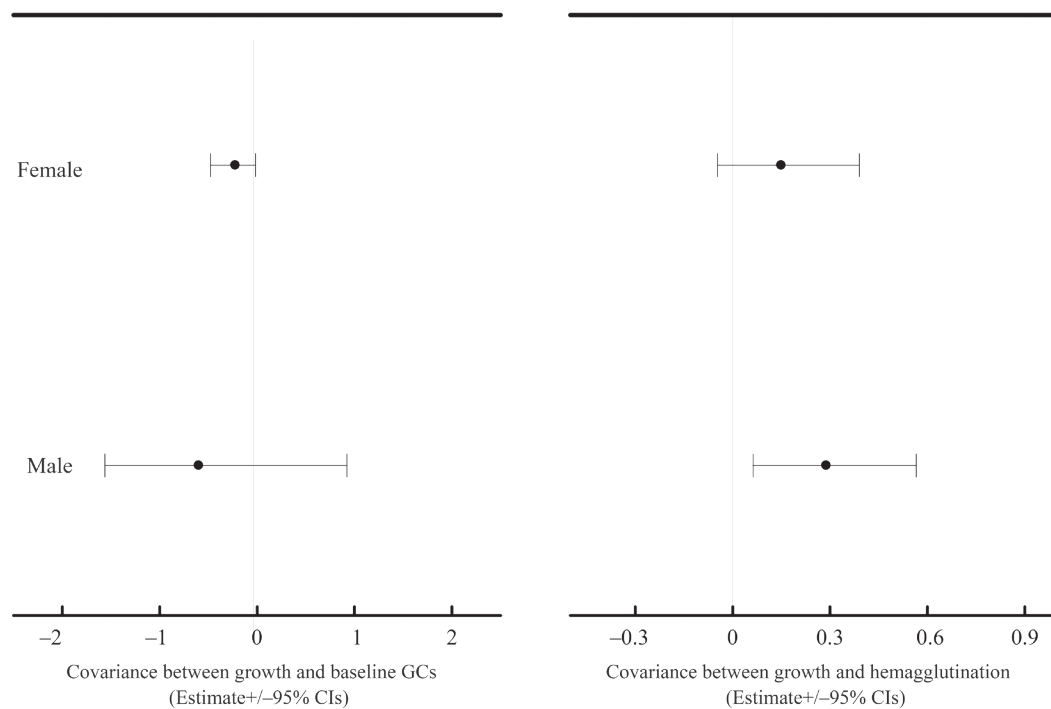


Figure 2 The estimate and 95% confidence interval for the covariances between growth rate and baseline GCs and hemagglutination for males and females of Dataset II.

The evolution of sexual dimorphism in POLS has been highlighted because of the obvious differences between the sexes in life history optima and consequently in the optimal expression of life history, behavioral and physiological traits involved in POLS (Immonen *et al.*, 2018). In *B. gargarizans*, we detected a negative covariation between growth rate and baseline GCs in females and a positive covariation between growth rate and hemagglutination in males at the within-population level; both of these results indicate a sex-specific POLS composition (i.e., the specific traits that form a POLS differ between the sexes). Considering the obvious sex-biased reproductive lifespan in *B. gargarizans*, these results support the predictions that sexual dimorphism in POLS is most likely to occur in species whose reproductive roles differ between the sexes (Immonen *et al.*, 2018; Hamalainen *et al.*, 2018). The enhanced covariation between growth and baseline GCs in female toads may help them to invest more resources in future reproduction, while the greater covariation between growth and hemagglutination in males suggest that they are likely to be more sensitive to parasite infections than females, which is potentially associated with sex-specific hormonal immunosuppression (Desprat *et al.*, 2015). As current phenotypic trait covariances may not accurately reflect the genetic covariances, further investigations need to address

the underlying genetic basis of these sex-specific POLS composition.

Overall, this study showed that adult *B. gargarizans* mainly displayed consistent differences in growth rate, metabolic, immunological and hormonal traits between sexes, but no altitude. Moreover, sex- but not altitude- modified the phenotypic integration between growth rate and hormonal and immunological traits at the within-population level. Our study demonstrates that the prevalence of sex-specific POLS and highlights its role in understanding the evolution, maintenance and variability of POLS.

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References

- Alcalay Y., Scharf I., Ovadia O. 2015. Foraging syndromes and trait variation in antlions along a climatic gradient. *Oecologia*, 178(4): 1093–1103
- Bengston S. E., Dornhaus A. 2015. Latitudinal variation in behaviors linked to risk tolerance is driven by nest-site competition and spatial distribution in the ant *Temnothorax rugatulus*. *Behav Ecol Sociobiol*, 69(8): 1265–1274
- Brans K. I., Stoks R., De Meester L. 2018. Urbanization drives genetic differentiation in physiology and structures the evolution of pace-of-life syndromes in the water flea *Daphnia magna*. *Proc R Soc Lond Ser B Biol Sci*, 285: 20180169
- Careau V., Thomas D., Humphries M. M., Réale D. 2008. Energy metabolism and animal personality. *Oikos*, 117(5): 641–653
- Chou C. C., Iwasa Y., Nakazawa T. 2016. Incorporating an ontogenetic perspective into evolutionary theory of sexual size dimorphism. *Evolution*, 70(2): 369–384
- Conover D. O., Duffy T. A., Hice L. A. 2009. The covariance between genetic and environmental influences across ecological gradients reassessing the evolutionary significance of countergradient and cgradient variation. *Ann N Y Acad Sci*, 1168: 100–129
- Conover D. O., Schultz E. T. 1995. Phenotypic Similarity and the Evolutionary Significance of Countergradient Variation. *Trends Ecol Evol*, 10(6): 248–252
- Crespi E. J., Williams T. D., Jessop T. S., Delehanty B. 2013. Life history and the ecology of stress: How do glucocorticoid hormones influence life-history variation in animals? *Funct Ecol*, 27(1): 93–106
- Dammhahn M., Dingemanse N. J., Niemela P. T., Reale D. 2018. Pace-of-life syndromes: A framework for the adaptive integration of behaviour, physiology and life history. *Behav Ecol Sociobiol*, 72: 62
- Davis A. K., Maney D. L. 2018. The use of glucocorticoid hormones or leucocyte profiles to measure stress in vertebrates: What's the difference? *Methods Ecol Evol*, 9(6): 1556–1568
- Debecker S., Stoks R. 2019. Pace of life syndrome under warming and pollution: Integrating life history, behavior, and physiology across latitudes. *Ecol Monogr*, 89(1): e01332
- Desprat J. L., Lengagne T., Dumet A., Desouhant E., Mondy N. 2015. Immunocompetence handicap hypothesis in tree frog: Trade-off between sexual signals and immunity? *Behav Ecol*, 26(4): 1138–1146
- Fei L., Hu S., Ye C., Huang Y. 2009. *Fauna Sinica, Amphibia*, Vol 2. Beijing: Science Press
- Folstad I., Karter A. J. 1992. Parasites, bright males, and the immunocompetence handicap. *Am Nat*, 139(3): 603–622
- Fournier P. A., Petrof E. O., Guderley H. 1992. The Glucosidic pathways and glucose-production by frog-muscle. *J Biol Chem*, 267(12): 8234–8237
- Guo B. C., Lu D., Liao W. B., Merila J. 2016. Genomewide scan for adaptive differentiation along altitudinal gradient in the Andrew's toad *Bufo andrewsi*. *Mol Ecol*, 25(16): 3884–3900
- Hadfield J. D. 2010. MCMC methods for multi-response generalized linear mixed models: The MCMCglmm R Package. *J Stat Softw*, 33(2): 1–22
- Hamalainen A., Immonen E., Tarka M., Schuett W. 2018. Evolution of sex-specific pace-of-life syndromes: Causes and consequences. *Behav Ecol Sociobiol*, 72: 50
- Immonen E., Hamalainen A., Schuett W., Tarka M. 2018. Evolution of sex-specific pace-of-life syndromes: Genetic architecture and physiological mechanisms. *Behav Ecol Sociobiol*, 72(3): 60
- Janin A., Lena J. P., Deblois S., Joly P. 2012. Use of stress-hormone levels and habitat selection to assess functional connectivity of a landscape for an amphibian. *Conserv Biol*, 26(5): 923–931
- Kim S. Y., Noguera J. C., Morales J., Velando A. 2011. Quantitative genetic evidence for trade-off between growth and resistance to oxidative stress in a wild bird. *Evol Ecol*, 25(2): 461–472
- Klingenberg C. P. 2014. Studying morphological integration and modularity at multiple levels: Concepts and analysis. *Philos T Roy Soc B*, 369: 1649
- Landys M. M., Ramenofsky M., Wingfield J. C. 2006. Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. *Gen Comp Endocrinol*, 148(2): 132–149
- Lankford T. E., Billerbeck J. M., Conover D. O. 2001. Evolution of intrinsic growth and energy acquisition rates. II. Trade-offs with vulnerability to predation in *Menidia menidia*. *Evolution*, 55(9): 1873–1881
- Liao W. B., Lu X. 2012. Adult body size = f (initial size plus growth rate x age): Explaining the proximate cause of Bergman's cline in a toad along altitudinal gradients. *Evol Ecol*, 26(3): 579–590
- Liao W. B., Lu X., Jehle R. 2014. Altitudinal variation in maternal investment and trade-offs between egg size and clutch size in the Andrew's toad. *J Zool*, 293(2): 84–91
- Lochmiller R. L., Deerenberg C. 2000. Trade-offs in evolutionary immunology: Just what is the cost of immunity? *Oikos*, 88(1): 87–98
- Londono G. A., Chappell M. A., Castaneda M. D., Jankowski J. E., Robinson S. K. 2015. Basal metabolism in tropical birds: Latitude, altitude, and the 'pace of life'. *Funct Ecol*, 29(3): 338–346
- Matson K. D., Ricklefs R. E., Klasing K. C. 2005. A hemolysis-hemagglutination assay for characterizing constitutive innate humoral immunity in wild and domestic birds. *Dev Comp Immunol*, 29(3): 275–286
- Montiglio P. O., Dammhahn M., Messier G. D., Réale D. 2018. The pace-of-life syndrome revisited: The role of ecological conditions and natural history on the slow-fast continuum. *Behav Ecol Sociobiol*, 72: 116
- Pettersen A. K. 2020. Countergradient variation in reptiles: Thermal sensitivity of developmental and metabolic rates across locally adapted populations. *Front Physiol*, 11: 547
- Polverino G., Santostefano F., Diaz-Gil C., Mehner T. 2018. Ecological conditions drive pace-of-life syndromes by shaping relationships between life history, physiology and behaviour in two populations of eastern mosquitofish. *Sci Rep*, 8: 14673
- Pruitt J. N., Riechert S. E., Jones T. C. 2008. Behavioural syndromes and their fitness consequences in a socially polymorphic spider, *Anelosimus studiosus*. *Anim Behav*, 76: 871–879
- Réale D., Garant D., Humphries M. M., Bergeron P., Careau V., Montiglio P. O. 2010. Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philos T Roy Soc B*, 365(1560): 4051–4063
- Reznick D., Nunney L., Tessier A. 2000. Big houses, big cars, superfleas and the costs of reproduction. *Trends Ecol Evol*, 15(10): 421–425
- Ricklefs R. E., Wikelski M. 2002. The physiology/life-history nexus. *Trends Ecol Evo*, 17(10): 462–468
- Seebacher F., Grigaltchik V. S. 2014. Embryonic developmental temperatures modulate thermal acclimation of performance curves in tadpoles of the frog *Limnodynastes peronii*. *PLoS One* 9(9): e106492
- Segev U., Burkert L., Feldmeyer B., Foitzik S. 2017. Pace-of-life in a social insect: Behavioral syndromes in ants shift along a climatic gradient. *Behav Ecol*, 28(4): 1149–1159
- Sparkman A. M., Palacios M. G. 2009. A test of life-history theories of

- immune defence in two ecotypes of the garter snake, *Thamnophis elegans*. *J Anim Ecol*, 78(6): 1242–1248
- Speakman J. R. 2005. Body size, energy metabolism and lifespan. *J Exp Biol*, 208 (Pt 9): 1717–1730
- Stearns S. C. 1989. Trade-offs in life-history evolution. *Funct Ecol*, 3(3): 259–268
- Tieleman B. I., Dijkstra T. H., Lasky J. R., Mauck R. A., Visser G. H., Williams J. B. 2006. Physiological and behavioural correlates of life-history variation: A comparison between tropical and temperate zone House Wrens. *Funct Ecol*, 20(3): 491–499
- Tieleman B. I., Williams J. B., Ricklefs R. E., Klasing K. C. 2005. Constitutive innate immunity is a component of the pace-of-life syndrome in tropical birds. *Proc R Soc Lond Ser B Biol Sci*, 272(1573): 1715–1720
- Tomasek O., Bobek L., Kralova T., Adamkova M., Albrecht T. 2019. Fuel for the pace of life: Baseline blood glucose concentration co-evolves with life-history traits in songbirds. *Funct Ecol*, 33(2): 239–249
- van Noordwijk A. J., de Jong G. 1986. Acquisition and allocation of resources: Their influence on variation in life history tactics. *Am Nat*, 128(1): 137–142
- Wedell N., Kvarnemo C., Lessells C. K. M., Tregenza T. 2006. Sexual conflict and life histories. *Anim Behav*, 71: 999–1011
- Werner E. E. 1986. Amphibian metamorphosis: Growth rate, predation risk, and the optimal size at transformation. *Am Nat*, 128(3): 319–341
- West-Eberhard M. J. 2005. Phenotypic accommodation: Adaptive innovation due to developmental plasticity. *J Exp Zool*, 304B: 610–618
- Wiersma P., Munoz-Garcia A., Walker A., Williams J. B. 2007. Tropical birds have a slow pace of life. *Proc Natl Acad Sci USA*, 104(22): 9340–9345

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Appendix

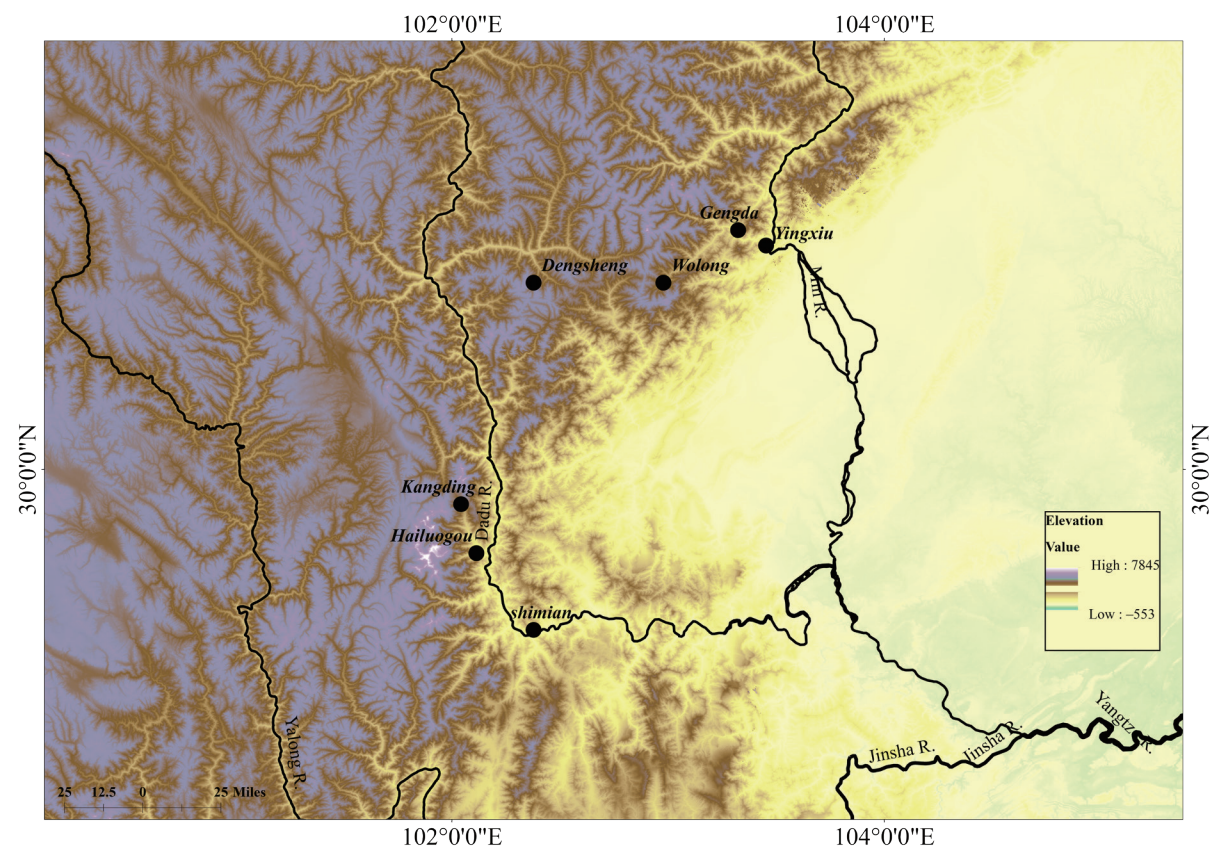


Figure S1 Sample sites of the study.

Table S1 Estimates of fixed effects and variance components for growth rate and physiological traits of dataset I, obtained from univariate mixed models. The table gives the mean posterior distribution and its 95% credible interval (CI).

	Estimate	L-95% CI	U-95% CI	pMCMC
Growth rate				
Intercept	2.044	0.382	0.375	0.017*
Altitude	-0.939	-2.726	0.714	0.24
Transect	-0.852	-3.17	1.375	0.37
Transect×Altitude	0.667	-1.766	3.491	0.573
Covariate (BM)	-0.032	-0.041	-0.023	<0.002**
Random effect	0.584	0.059	1.686	
Residual	0.485	0.355	0.636	
Blood glucose				
Intercept	-0.386	-2.67	2.9	0.753
Altitude	-0.288	-2.536	1.383	0.68
Transect	-1.663	-4.21	0.288	0.1
Transect×Altitude	0.469	-2.114	3.475	0.64
Covariate (SVL)	0.014	-0.014	0.04	0.287
Random effect	0.68	0.063	1.831	
Residual	0.486	0.346	0.663	
Baseline GCs				
Intercept	0.545	-2.618	3.397	0.723
Altitude	0.064	-2.048	1.82	0.923
Transect	1.063	-1.222	3.188	0.26
Transect×Altitude	-0.695	-3.376	1.752	0.563
Covariate (SVL)	-0.006	-0.038	0.027	0.693
Random effect	0.624	0.076	1.796	
Residual	0.731	0.513	0.98	
Stress-induced GCs				
Intercept	4.396	1.533	7.96	0.007**
Altitude	-0.493	-2.525	1.954	0.573
Transect	0.461	-1.813	3.106	0.667
Transect×Altitude	-0.603	-3.191	2.002	0.613
Covariate (SVL)	-0.052	-0.081	-0.02	0.003**
Random effect	0.825	0.0845	2.359	
Residual	0.724	0.514	0.989	
Hemolysis				
Intercept	0.577	-2.806	3.546	0.757
Altitude	-0.475	-2.548	1.58	0.597
Transect	-0.3	-2.654	2.571	0.707
Transect×Altitude	-0.14	-3.644	2.634	0.897
Covariate (SVL)	-0.003	-0.037	0.029	0.843
Random effect	0.68	0.07	1.995	
Residual	0.772	0.527	1.049	
Hemagglutination				
Intercept	3.172	0.061	6.515	0.05
Altitude	-0.382	-2.225	1.616	0.66
Transect	0.048	-2.386	2.242	0.963
Transect×Altitude	-1.091	-3.938	1.693	0.377
Covariate (SVL)	-0.036	-0.069	-0.006	0.03
Random effect	0.699	0.074	2.229	
Residual	0.733	0.53	0.975	

Table S2 Estimates of fixed effects and variance components for growth rate and physiological traits of dataset II, obtained from univariate mixed models. The table gives the mean posterior distribution and its 95% credible interval (CI).

	Estimate	L-95% CI	U-95% CI	pMCMC
Growth rate				
Intercept	2.235	0.431	3.733	<0.001**
Altitude	-0.82	-3.361	1.426	0.347
Sex	-1.674	-2.137	-1.242	<0.002**
Sex×Altitude	0.423	-0.232	1.083	0.203
Covariate (BM)	-0.014	-0.018	-0.009	<0.002**
Random effect	1.386	0.052	4.165	
Residual	0.576	0.411	0.781	
Blood glucose				
Intercept	0.92	-1.583	3.097	0.433
Altitude	-0.049	-1.876	2.067	0.897
Sex	-0.051	-0.557	0.553	0.857
Sex×Altitude	-0.21	-0.871	0.541	0.55
Covariate (SVL)	-0.004	-0.024	0.016	0.71
Random effect	1.071	0.083	3.574	
Residual	0.637	0.426	0.84	
Baseline GCs				
Intercept	-3.268	-5.677	-0.267	0.017*
Altitude	0.521	-1.763	3.155	0.583
Sex	1.126	0.521	1.678	0.003**
Sex×Altitude	0.176	-0.525	0.926	0.65
Covariate (SVL)	0.026	0.005	0.048	0.013*
Random effect	1.434	0.097	4.538	
Residual	0.712	0.506	0.91	
Stress-induced GCs				
Intercept	2.686	0.028	4.694	0.017*
Altitude	-0.07	-2.086	1.904	0.986
Sex	0	-0.602	0.544	0.893
Sex×Altitude	0.209	-0.498	1.033	0.603
Covariate (SVL)	-0.033	-0.052	-0.011	<0.002**
Random effect	1.14	0.06	3.836	
Residual	0.746	0.533	0.956	
Hemolysis				
Intercept	2.816	0.596	5.461	0.027*
Altitude	-0.113	-0.21	1.969	0.86
Sex	-0.66	-1.195	-0.113	0.017*
Sex×Altitude	-0.319	-1.027	0.422	0.407
Covariate (SVL)	-0.025	0.048	-0.006	0.020*
Random effect	1.064	0.065	3.559	
Residual	0.66	0.483	0.889	
Hemagglutination				
Intercept	3.842	0.851	6.227	0.017*
Altitude	-0.678	-3.365	1.857	0.49
Sex	-0.773	-1.381	-0.153	0.033*
Sex×Altitude	0.324	-0.33	0.994	0.383
Covariate (SVL)	-0.037	-0.063	-0.015	<0.002**
Random effect	1.738	0.094	5.506	
Residual	0.747	0.533	1.005	

Table S3 Estimates of fixed effects and variance components for growth rate and physiological traits in of dataset I, obtained from bivariate mixed models. Panel A reports the estimates of fixed effects and variance components only with covariates included in the explanatory variables, and panel B reports the variance components after including altitude into the explanatory variables. Panel C reports the variance components after including transect into the explanatory variables. Variation in response and explanatory variables were scaled before analysis to facilitate the interpretation of the estimates. Given the structure of the data, the residual variance and covariance are interpreted as the phenotypic (co)variance within populations. The table gives the mean posterior distribution and its 95% credible interval (CI).

	Fixed effects	Growth rate			Blood glucose		
		Estimate	95% CI	<i>P</i>	Estimate	95% CI	<i>P</i>
Panel A	Intercept	−0.009	−0.454, 0.392	0.974	−0.002	−0.936, 0.920	0.964
	Body mass/SVL	−0.211	−0.470, 0.067	0.136	−0.047	−0.291, 0.165	0.633
	Var _{population}	0.244	0, 0.876		1.364	0.095, 3.791	
	Var _{residual}	0.944	0.681, 1.319		0.576	0.419, 0.786	
	Covar _{population}	0.22	−0.310, 0.840				
	Covar _{residual}	−0.009	−0.202, 0.178				
Panel B	Var _{population}	0.316	0, 1.065		1.933	0.124, 5.714	
	Var _{residual}	0.962	0.690, 1.368		0.583	0.390, 0.767	
	Covar _{population}	0.239	−0.561, 1.222				
	Covar _{residual}	−0.01	−0.220, 0.217				
Panel C	Var _{population}	0.167	0, 0.636		0.251	0, 0.984	
	Var _{residual}	0.962	0.683, 1.320		0.58	0.404, 0.788	
	Covar _{population}	−0.003	−0.140, 0.213				
	Covar _{residual}	−0.043	−0.272, 0.150				
	Fixed effects	Growth rate			Baseline GCs		
		Estimate	95% CI	<i>P</i>	Estimate	95% CI	<i>P</i>
Panel A	Intercept	−0.007	−0.372, 0.367	0.954	0.004	−0.408, 0.466	0.992
	Body mass/SVL	0.105	−0.163, 0.346	0.462	−0.16	−0.376, 0.101	0.249
	Var _{population}	0.131	0, 0.502		0.25	0, 0.772	
	Var _{residual}	0.963	0.669, 1.301		−0.334	−0.610, −0.100	
	Covar _{population}	−0.083	−0.341, 0.101				
	Covar _{residual}	−0.334	−0.610, −0.100				
Panel B	Var _{population}	0.242	0, 0.746		0.398	0, 1.258	
	Var _{residual}	0.967	0.673, 1.317		0.939	0.617, 1.248	
	Covar _{population}	−0.118	−0.565, 0.165				
	Covar _{residual}	−0.335	−0.609, −0.074				
Panel C	Var _{population}	0.129	0, 0.383		0.115	0, 0.406	
	Var _{residual}	0.962	0.678, 1.305		0.91	0.633, 1.202	
	Covar _{population}	−0.017	−0.147, 0.068				
	Covar _{residual}	−0.31	−0.588, −0.078				
	Fixed effects	Growth rate			Stress-induced GCs		
		Estimate	95% CI	<i>P</i>	Estimate	95% CI	<i>P</i>
Panel A	Intercept	−0.008	−0.412, 0.388	0.987	−0.048	−0.355, 0.406	0.777
	Body mass/SVL	−0.197	−0.467, 0.108	0.159	−0.173	−0.431, 0.071	0.2
	Var _{population}	0.212	0, 0.728		0.185	0, 0.658	
	Var _{residual}	0.968	0.650, 1.313		0.944	0.651, 1.253	
	Covar _{population}	−0.037	−0.278, 0.158				
	Covar _{residual}	0.004	−0.266, 0.252				
Panel B	Var _{population}	0.316	0, 1.240		0.267	0, 0.828	
	Var _{residual}	0.967	0.691, 1.287		0.958	0.667, 1.307	
	Covar _{population}	−0.054	−0.453, 0.216				
	Covar _{residual}	0.038	−0.274, 0.300				
Panel C	Var _{population}	0.156	0, 0.561		0.277	0, 1.091	
	Var _{residual}	0.958	0.651, 1.289		0.964	0.698, 1.297	
	Covar _{population}	−0.003	−0.155, 0.281				
	Covar _{residual}	0.009	−0.250, 0.245				

Continued Table S3

	Fixed effects	Growth rate			Hemolysis		
		Estimate	95% CI	<i>P</i>	Estimate	95% CI	<i>P</i>
Panel A	Intercept	−0.009	−0.416, 0.379	0.977	−0.033	−0.424, 0.311	0.882
	Body mass/SVL	−0.186	−0.442, 0.056	0.156	−0.157	−0.418, 0.101	0.279
	Var _{population}	0.173	0, 0.656		0.144	0, 0.566	
	Var _{residual}	0.981	0.688, 1.349		1.003	0.697, 1.352	
	Covar _{population}	0.043	−0.114, 0.252				
	Covar _{residual}	0.155	−0.093, 0.438				
Panel B	Var _{population}	0.311	0, 0.993		0.261	0, 1.018	
	Var _{residual}	0.957	0.689, 1.302		1.018	0.704, 1.378	
	Covar _{population}	0.057	−0.196, 0.372		0.156	−0.141, 0.433	
	Covar _{residual}						
Panel C	Var _{population}	0.122	0, 0.533		0.16	0, 0.666	
	Var _{residual}	0.957	0.689, 1.293		0.992	0.687, 1.325	
	Covar _{population}	0.014	−0.134, 0.178				
	Covar _{residual}	0.122	−0.111, 0.390				
	Fixed effects	Growth rate			Hemagglutination		
		Estimate	95% CI	<i>P</i>	Estimate	95% CI	<i>P</i>
Panel A	Intercept	−0.015	−0.396, 0.343	0.943	−0.12	−0.700, 0.482	0.708
	Body mass/SVL	−0.181	−0.431, 0.061	0.128	−0.276	−0.576, −0.039	0.046
	Var _{population}	0.197	0, 0.642		0.591	0, 1.785	
	Var _{residual}	0.964	0.681, 1.291		0.881	0.606, 1.193	
	Covar _{population}	0.101	−0.235, 0.480				
	Covar _{residual}	0.256	0.038, 0.533				
Panel B	Var _{population}	0.306	0, 1.082		0.821	0, 2.845	
	Var _{residual}	0.965	0.682, 1.291		0.866	0.603, 1.205	
	Covar _{population}	0.099	−0.570, 0.608				
	Covar _{residual}	0.273	0.046, 0.532				
Panel C	Var _{population}	0.176	0, 0.612		0.614	0, 2.023	
	Var _{residual}	0.944	0.678, 1.262		0.859	0.618, 1.153	
	Covar _{population}	0.043	−0.237, 0.394				
	Covar _{residual}	0.245	0.016, 0.497				

Table S4 Estimates of fixed effects and variance components for growth rate and physiological traits in of dataset II, obtained from bivariate mixed models. Panel A reports the estimates of fixed effects and variance components only with covariates included in the explanatory variables, and panel B reports the variance components after including altitude into the explanatory variables. Panel C reports the variance components after including transect into the explanatory variables. Variation in response and explanatory variables were scaled before analysis to facilitate the interpretation of the estimates. Given the structure of the data, the residual variance and covariance are interpreted as the phenotypic (co)variance within populations. The table gives the mean posterior distribution and its 95% credible interval (CI).

	Fixed effects	Growth rate			Blood glucose		
		Estimate	95% CI	<i>P</i>	Estimate	95% CI	<i>P</i>
Panel A	Intercept	−0.075	−0.910, 0.576	0.846	0.019	−0.848, 0.818	0.969
	Body mass/SVL	0.038	−0.189, 0.293	0.772	−0.206	−0.421, 0.032	0.068
	Var _{population}	0.884	0, 3.281		1.614	0, 2.583	
	Var _{residual}	0.995	0.715, 1.351		1.022	0.698, 1.326	
	Covar _{population}	−0.042	−0.650, 0.794				
	Covar _{residual}	0.091	−0.101, 0.374				
Panel B	Var _{population}	34.006	0, 51.394		12.912	0, 40.683	
	Var _{residual}	0.99	0.673, 1.300		1.011	0.724, 1.369	
	Covar _{population}	2.096	−4.817, 6.125		0.106	−0.162, 0.341	
	Covar _{residual}						
Panel C	Var _{population}	2.668	0, 8.771		3.36	0, 2.882	
	Var _{residual}	0.558	0.382, 0.722		1.016	0.704, 1.335	
	Covar _{population}	0.15	−0.107, 1.502				
	Covar _{residual}	0.023	−0.131, 0.209				
	Fixed effects	Growth rate			Baseline GCs		
		Estimate	95% CI	<i>P</i>	Estimate	95% CI	<i>P</i>
Panel A	Intercept	−0.034	−0.618, 0.390	0.885	0.039	−0.497, 0.599	0.892
	Body mass/SVL	−0.17	−0.392, 0.047	0.146	0.023	−0.204, 0.256	0.849
	Var _{population}	0.962	0, 1.421		0.641	0, 1.542	
	Var _{residual}	0.997	0.713, 1.313		1.043	0.716, 1.399	
	Covar _{population}	−0.062	−0.545, 0.245				
	Covar _{residual}	−0.568	−0.846, −0.323				
Panel B	Var _{population}	21.316	0, 27.827		44.385	0, 48.555	
	Var _{residual}	0.998	0.673, 1.339		1.047	0.700, 1.404	
	Covar _{population}	−0.763	−4.55, 4.684				
	Covar _{residual}	−0.568	−0.870, −0.299				
Panel C	Var _{population}	1.167	0, 4.052		1.188	0, 4.654	
	Var _{residual}	0.562	0.377, 0.752		0.813	0.577, 1.094	
	Covar _{population}	−0.258	−1.447, 1.037				
	Covar _{residual}	−0.247	−0.426, −0.081				
	Fixed effects	Growth rate			Stress-induced GCs		
		Estimate	95% CI	<i>P</i>	Estimate	95% CI	<i>P</i>
Panel A	Intercept	−0.06	0.841, 0.793	0.841	−0.214	−0.437, 0.040	0.069
	Body mass/SVL	−0.214	−0.438, 0.040	0.069	−0.436	−0.656, −0.195	<0.001*
	Var _{population}	1.707	0, 3.059		0.8	0, 1.990	
	Var _{residual}	0.995	0.686, 1.307		0.826	0.594, 1.093	
	Covar _{population}	0.017	−0.811, 0.424				
	Covar _{residual}	−0.144	−0.366, 0.077				
Panel B	Var _{population}	23.906	0, 71.95		33.952	0, 28.59	
	Var _{residual}	1	0.723, 1.352		0.827	0.583, 1.102	
	Covar _{population}	0.2	−5.77, 14.42				
	Covar _{residual}	−0.133	−0.390, 0.090				
Panel C	Var _{population}	2.151	0, 6.898		1.757	0, 2.625	
	Var _{residual}	0.561	0.387, 0.762		0.838	0.587, 1.034	
	Covar _{population}	−0.047	−1.052, 0.974				
	Covar _{residual}	−0.101	−0.253, 0.082				

Continued Table S4

	Fixed effects	Growth rate			Hemolysis		
		Estimate	95% CI	<i>P</i>	Estimate	95% CI	<i>P</i>
Panel A	Intercept	−0.056	−0.881, 0.677	0.872	−0.195	−0.394, 0.051	0.108
	Body mass/SVL	−0.194	−0.394, 0.051	0.108	−0.06	−0.305, 0.174	0.664
	Var _{population}	1.008	0, 3.088		0.596	0, 0.997	
	Var _{residual}	0.981	0.679, 1.286		1.056	0.748, 1.379	
	Covar _{population}	0.041	−0.335, 0.407				
	Covar _{residual}	0.232	−0.015, 0.468				
Panel B	Var _{population}	36.505	0, 29.876		10.698	0, 24.306	
	Var _{residual}	0.981	0.689, 1.293		1.076	0.755, 1.441	
	Covar _{population}	0.115	−2.554, 6.202				
	Covar _{residual}	0.243	0, 0.505				
Panel C	Var _{population}	7.225	0.002, 8.672		0.851	0, 3.259	
	Var _{residual}	0.557	0.355, 0.730		0.961	0.661, 1.283	
	Covar _{population}	0.149	−1.153, 1.378				
	Covar _{residual}	0.025	−0.156, 0.198				
	Fixed effects	Growth rate			Hemagglutination		
		Estimate	95% CI	<i>P</i>	Estimate	95% CI	<i>P</i>
Panel A	Intercept	−0.029	−0.786, 0.736	0.88	−0.063	−0.952, 0.997	0.846
	Body mass/SVL	−0.207	−0.448, 0	0.072	−0.421	0.661, −0.197	<0.001*
	Var _{population}	0.653	0, 2.354		1.475	0, 4.931	
	Var _{residual}	0.996	0.697, 1.280		0.962	0.652, 1.303	
	Covar _{population}	0.083	−0.586, 1.092				
	Covar _{residual}	0.472	0.230, 0.764				
Panel B	Var _{population}	10.061	0, 42.757		24.355	0, 61.043	
	Var _{residual}	0.983	0.691, 1.300		0.93	0.639, 1.244	
	Covar _{population}	0.529	−6.136, 8.267				
	Covar _{residual}	0.448	0.204, 0.727				
Panel C	Var _{population}	1.962	0, 6.574		2.597	0, 8.527	
	Var _{residual}	0.554	0.363, 0.753		0.863	0.599, 1.160	
	Covar _{population}	0.392	−1.352, 3.222				
	Covar _{residual}	0.26	0.083, 0.473				